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## Changes in milk yield, lactate dehydrogenase, milking frequency, and interquarter yield ratio persist for up to 8 weeks after antibiotic treatment of mastitis

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### ABSTRACT

Within the dairy industry, the appearance of milk and withdrawal time due to antibiotic residuals in the milk are used to determine recovery status after cases of treated mastitis. However, both milk production and dairy cow behavior have been shown to be affected after the normalization of milk appearance, indicating that animals may not have fully recovered. The aim of the present study was to describe the changes in milk yield, lactate dehydrogenase activity, milking frequency, and interquarter yield ratio (defined as the coefficient of variation between the active quarters) after cases of naturally occurring mastitis with special focus on the recovery period after antibiotic treatment. A second aim was to examine whether these changes were affected by the pathogens present at the time of mastitis diagnosis. This retrospective study was based on a cohort data set including 1,032 lactations from 795 dairy cows kept on 2 Danish farms and milked by an automatic milking system. A total of 174 treated mastitis cases were compared with nontreated control cows from 5 wk before treatment and until 8 wk after. Treated mastitis resulted in reduced milk yield, elevated lactate dehydrogenase activity, lower milking frequency, and elevated interquarter yield ratio. Within these measures, deviations from baseline levels and from the control cows were found as early as 1 to 3 wk before the antibiotic treatment and peaked around the days of treatment. In some cases, the mastitic cows returned to premastitis levels, whereas in others they remained affected throughout the rest of the observation period. To correctly estimate the effects of treated mastitis and the recovery status of cows, it is important to take the individual cow into account and not only compare

with herd levels, as this might mask the true degree of the changes. The effects on each outcome variable depended on the involved pathogen and differences were found between primiparous cows and older animals. However, in general, the changes in milk production, lactate dehydrogenase activity, and interquarter yield ratio showed parallels, suggesting that the recovery period continued for weeks after antibiotic treatment. These results call for further investigation into management of mastitic dairy cows to optimize recovery, limit milk loss, and ensure animal welfare during the period after mastitis.

**Key words:** dairy cow, mastitis, recovery, automatic milking system, lactate dehydrogenase

### INTRODUCTION

For decades, research has focused on early identification of dairy cows with mastitis. Several diagnostic tools have been developed and can be combined in mastitis-control programs (Rutten et al., 2013). The recovery period following mastitis has received less scientific attention. Within the dairy industry, the appearance of the milk and lack of antibiotic residuals in the milk are used to determine recovery after cases of naturally occurring mastitis. However, previous studies have shown that cows have elevated SCC and decreased milk production at least 8 wk after mastitis (Gröhn et al., 2004; Schukken et al., 2009a; Hertl et al., 2014). Hence, data already available on-farm in the modern dairy industry may reveal new insights into recovery after mastitis; however, at present, this phase is not well understood.

Analyses of SCC in composite milk samples (milk mixed from all active quarters) are most commonly used as an indicator of mastitis (Nyman et al., 2014), but other udder health indicators also exist, such as inline measurements of lactate dehydrogenase (LDH). The LDH enzyme is part of the glycolytic pathway, present in the cytoplasm of all cells. During udder infection, the activity of LDH in composite milk increases as a result

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of disrupted udder tissue (Bogin et al., 1976). This increase has been shown to be a responsive indicator of mastitis and is currently being used for early detection of bovine mastitis (Chagunda et al., 2006b; Nyman et al., 2014).

Most cases of mastitis occur at quarter level, whereas most of the present diagnostic tools focus on cow level. Use of the interquarter ratio (IQR) of milk yield may be one way to estimate the level of mastitis at quarter level. The IQR is the coefficient of variation between the active quarters. This is supported by the finding that the IQR of electric conductivity can successfully distinguish between healthy and clinically infected cows (Norberg et al., 2004). Hence, the IQR of yield might serve as a valid measure of the ability of the udder to reach a stable level of milk production after a mastitis incident. If so, large IQR variation and fluctuations in the IQR variation may indicate lack of recovery after mastitis.

Cows milked by automatic milking systems (AMS) have some possibility to change their milking frequency, which would be expected during times of disease as a result of discomfort and malaise. During mastitis, being milked might be an aversive experience due to soreness of the udder (Fitzpatrick et al., 1998; Leslie et al., 2010), thus should be delayed or even avoided, if possible. Hence, in housing systems using AMS, the magnitude and duration of changes in milking frequency might contain information about the mastitis status of individual cows.

Here we report results from a retrospective study of 174 cases of treated mastitis from 2 Danish herds using AMS to achieve insights into the recovery phase after naturally occurring mastitis treated with antibiotics. We aimed to describe the changes in milk yield, LDH activity, milking frequency, and IQR of yield in primiparous and multiparous cows. A second aim was to examine whether these changes were affected by the mastitis-causing pathogens present at the time of diagnosis: *Staphylococcus aureus*, CNS, and *Escherichia coli*.

We hypothesized that several of the milk production-related traits connected to the function of the udder would be affected by mastitis—particularly the traits connected to the infected quarter(s). After antibiotic treatment, the response to mastitis was expected to differ in these 2 groups of cows and also for the types of pathogens involved. Based on previous research (Gröhn et al., 2004; Schukken et al., 2009a; Hertl et al., 2014) and the differences in the lactation curves of primiparous and multiparous cows (Wood, 1969), the response to treated mastitis in these 2 groups of cows was expected to differ and similarly for the types of pathogens involved.

## MATERIALS AND METHODS

### Design, Herds, and Animals

This study was designed as a retrospective cohort study (Ersbøll et al., 2004) with multivariate, irregularly spaced time series data. The initial data set included records from dairy cows <305 DIM and parity 1 to 3, recorded on 2 farms (Jutland, Denmark) from January 2010 (farm A) and November 2012 (farm B) to July 2014. Cows in parity 4 and later were excluded. Both farms were freestall dairy herds with AMS. Farm A milked an average of 210 dairy cows, both Jersey and Holstein-Friesian, in 3 milking units (on average  $2.9 \pm 0.8$  milkings/cow per day) and fed a balanced TMR in automatic feeding bins (Ric Systems, Insentec, Marknesse, the Netherlands). Farm B milked an average of 260 Holstein-Friesians in 4 milking units (on average  $2.8 \pm 0.8$  milkings/cow per day), and cows were fed a balanced TMR via feed alleys. On both farms, cows were supplied with concentrates in restricted amounts while being milked. The feed ration provided during any milking was controlled by the settings of the AMS software and based on time since last milking. On both farms, AMS had free cow traffic with the following settings of the robot: cows  $\leq 150$  DIM could only be milked with a minimum interval of 5 h or 7 kg of milk per milking, whereas cows  $>150$  DIM were allowed to be milked with an interval of minimum 8 h or 8 kg of milk per milking. The AMS software (DeLaval, Tumba, Sweden) calculated the milking interval of individual cows based on information from previous milkings and DIM. Irrespective of the DIM, cows with a milking interval exceeding 15 h were fetched for milking by barn staff twice a day.

### Outcome Variables

The 4 response variables of interest were milk yield, LDH activity, milking frequency, and the IQR of yield. Daily milk production at udder and quarter level (for quarter level only on farm A) was recorded automatically by the AMS. The 24-h milk yield (kg/d) was calculated as a 3-d moving average yield per 24 h, as suggested by ICAR (2011), taking yield per milking and milking frequency into account. This was done to reduce the effect of randomly occurring values for a particular milking. For the daily milking frequency, the mean number of milkings for the last 3 d was used to take the last 72 h into account. All milkings, including those where the cows had been fetched for milking, were included in the data.

On both farms, milk samples for LDH activity ( $\mu\text{mol}/\text{min}$  per liter) were taken automatically during

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